DIFFERENTIATION OF THE TRUNK OF THE LARVA IN THE ASCIDIANS, HALOCYNTHIA RORETZI AND BOTRYLLOIDES VIOLACEUS WITHOUT RESORPTION OF THE TAIL

BULLETIN OF THE MARINE BIOLOGICAL STATION OF ASAMUSHI,
VOL. XIV, NO. 1, 1970

DIFFERENTIATION OF THE TRUNK OF THE LARVA IN THE ASCIDIANS, *HALOCYNTHIA RORETZI* AND *BOTRYLLOIDES VIOLACEUS* WITHOUT RESORPTION OF THE TAIL[1)]

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In the ascidians *Halocynthia roretzi*, *Ciona intestinalis* and *Polycitor* sp., the body of the larva proceeded its metamorphosis with the disturbed and unresorbed tail by treatment with the lower temperature. But differentiation of the trunk is disturbed before long, though differences according to species was observed (Hirai, 1963). In the treatment with the low temperature on the larva of *Halocynthia roretzi*, the ampulla can be differentiated independently of the resorption of the tail in some larvae. When the ampulla was well differentiated, perhaps the adhesive papilla may disappear. In such larva with differentiated ampulla, further proceedings of the differentiation of the metamorphosis was disturbed even by the stimulation of the solution of Nile blue (Hirai, 1966). In 1968, Numakunai of the station obtained the larvae of *Halocynthia roretzi* which had further differentiated trunk with the unresorbed tail by treatment with MS-222 (Sankyo) (unpublished). The larvae of *Botrylloides violaceus* which kept swimming by the unresorbed tails with differentiated trunks were obtained. Some considerations on those larvae with unresorbed tails are mentioned in the present article.

MATERIALS AND METHOD

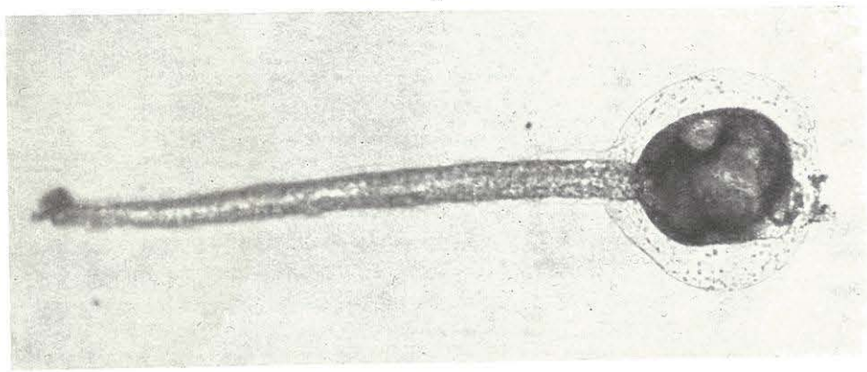
The larvae of *Halocynthia roretzi* were obtained from the spontaneously spawned eggs in the laboratory from November to December in 1968. The larvae of *Botrylloides violaceus* liberated from the colony in the laboratory from July to August. Numakunai treated the larvae of *H. roretzi* 12 hours after hatching by 10^{-7} gr per 1 ml of methanesulphonate of meta-aminobenzoic acid ethylester (abbreviated below MS-222. A commercial name of narcotics of Sankyo Ltd. Co.), and obtained larvae with further differentiated trunks though the tails were not resorbed. Those larvae were fixed with 10 per cent formlain, stained by borax-carmine and the total materials were observed in the solution of formalin mixed with some glycerin.

1) Contributions from the Marine Biological Station, Tôhoku University, Aomori City, No. 378

OBSERVATION

The larva of H. roretzi treated by MS-222. In 1968, Numakunai by treatment of the larva of *H. roretzi* with MS-222 obtained interesting results. The larvae 12 hours after hatching were kept in the sea water solution of MS-222. Active movements of the tails of the larvae stopped soon after they were placed in the solution. In the larvae which were kept in the MS-222 solution for about 13 days, further differentiated organs of the trunk were observed without the resorption of the tail (Fig. 1). In those larvae, the contracted anterior part of the trunk, the branchial and atrial apertures, the endostyle, a pair of gill slits, a canal of the

a



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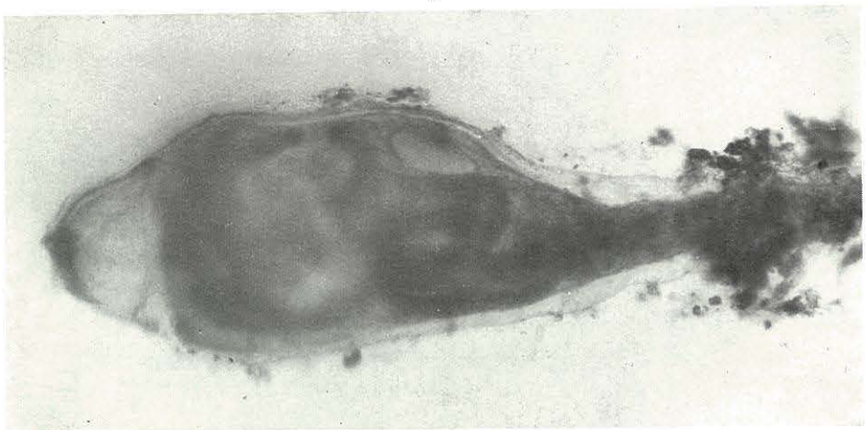


Fig. 1. Proceeded differentiation of the trunk without tail resorption in the larva of *Halocynthia roretzi*. a, A larva treated by low temperature (Hirai, 1963). Seventeen days after spawning. ×ca. 70 b, A larva treated by MS-222. Thirteen days after spawning. ×ca. 100

rudiment of the alimentary organs which appeared from the posterior end of the branchial sac, and in the living materials heart beating under oesophagus were observed. The canal of the rudiment of the alimentary organs occupies the posterior part of the larva, therefore the the rudiment of the branchial sac and alimentary canal are arranged along the antero-posterior axis of the larva. Further differentiated organs were observed in the trunks in such larvae than in the ones which were treated by the low temperature.

The larva of Botrylloides violaceus without resorption of tail. In *B. violaceus*, the larvae which showed progressed metamorphosis in the trunk without the resorption of the tail in the normal sea water (about 18°C) were obtained. In the present observation, 17 among the 52 larvae kept active swimming without resorption of the tail, though the well opened ampullae, well developed branchial and atrial siphones were visible in the trunk. Two or three days after the liberation, when those larvae with the unresorbed tail were stimulated by the solution of Nile blue (1% Nile blue 1 drop in 10 cc sea-water) for five minutes, the tail started resorption (Fig. 3). But in some of them the tails never showed resorption by the stimulation. In those larvae, no changes of the epidermis of the anterior parts of the tails were observed.

CONSIDERATION

In the metamorphosis of the larva of *H. roretzi*, the tail resorption is disturbed by the treatment with low temperature and by the solution of MS-222. When the larvae are treated by the low temperature, the differentiation of the trunk proceeded not so distinctly as with the larvae treated by MS-222 (Fig.1). In the larva treated by low temperature, the swimming movement of the tail is not disturbed, and the tunic is more differentiated than in the larva treated by MS-222. In the larva

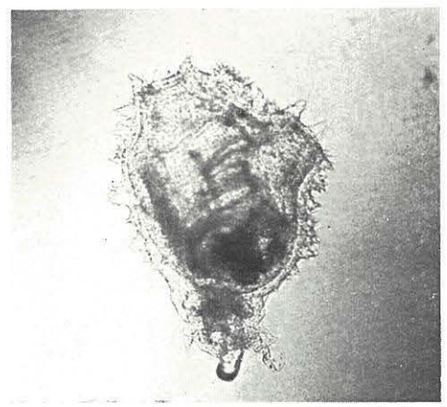
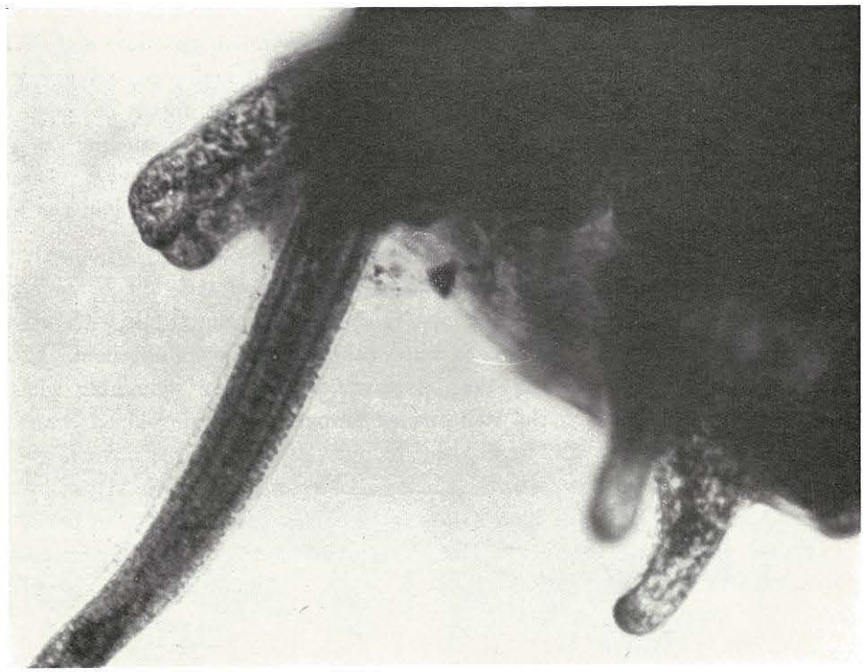


Fig. 2. Metamorphosed larva of *H. roretzi* 11 days after spawning in the control. ×45

a



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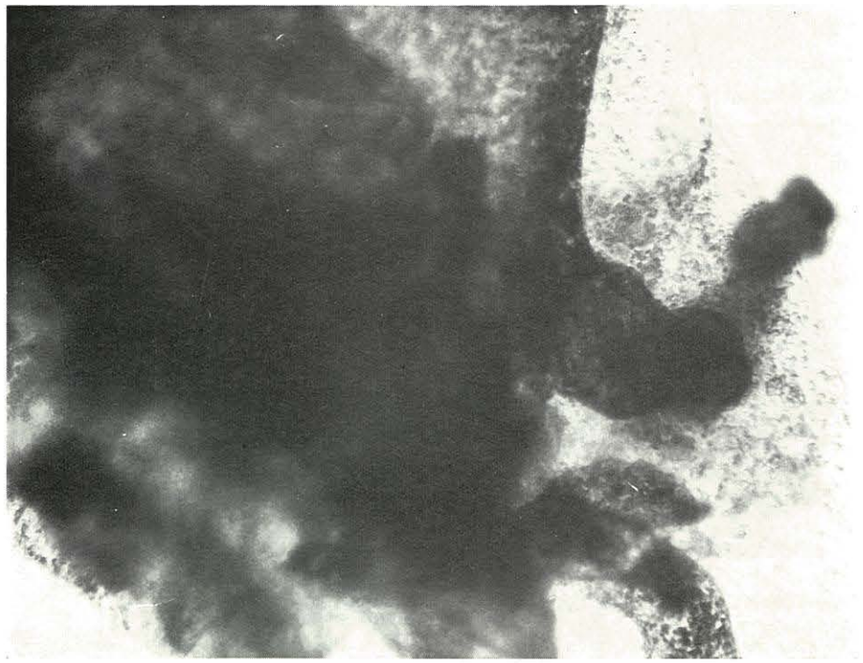

Fig. 3. Tail resorption of a larva of *Botrylloides violaceus* in which the trunk differentiated without tail resorption, by the stimulation of the solution of Nile blue. a, A tail of a larva keeping active swimming three days after liberation. ×ca. 60 b, Tail resorption of the same larve with (a) two hours after it was treated by the solution. ×ca. 60

treated by MS-222, further differentiated inner organs, branchial organs, alimentary organs and the heart were observed. But when this larva was compared with the control 11 days after spawning (Fig. 2), the disturbance of differentiations of all the organs were visible. The tunic is disturbed distinctly. The alimentary canal became differentiated, but was arranged at the more dorsal part in the larval antero-posterior axis compared with the control. It is supposed that this difference in arrangement of the organs is caused by the rotation or reorientation of the structures in the process of change from the larval organization to the adult organization is disturbed by the unresorbed tail. On this opinion it is supposed that the larva of *H. roretzi* is able to differentiate without tail resorption to a higher degree, but the further differentiation for the adult structure depends on the metamorphosis of the larval structure.

In *Amaroucium constellatum* numerous cases have been observed in which metamorphosis proceeded in the trunk without the resorption of the tail, and this depends on the spontaneous rupturing of the caudal epidermis (Cloney, 1963). In the case of *Polycitor* sp., the larva in which proceeded metamorphosis in the trunk without tail resorption, commenced resorption of the tail by the stimulation of Nile blue (Hirai, 1963), though the resorption was not sufficient. In the case of the larva of *Botrylloides violaceus* most of the larva in which proceeded metamorphosis in the trunk without tail resorption commenced resorption by the stimulation of Nile blue. In some larvae in which there was no resorption by the stimulation, no changes of the caudal epidermis were observed, and it was supposed that this fact perhaps depends on the change of structure of the adhesive papillae which was caused by the well opened ampullae of the larva as in the case of *H. roretzi*.

SUMMARY

(1) When the larvae of *Halocynthia roretzi* were treated by low temperature and by the solution of MS-222, the larvae in which proceeded metamorphosis in the trunks without tail resorption were obtained. The further differentiated organs of the trunk were observed in the larvae treated by MS-222 better than in those treated by the low temperature, but even in those larvae the disturbance for the differentiation of the trunk which was considered to be caused by the disturbed metamorphosis of the larval structure was observed.

(2) In *Botrylloides violaceus*, the larvae in which the proceeded differentiation in the trunk without tail resorption were obtained without any treatment. In those larvae, the tails could commence resorption by the stimulation of Nile blue with some exceptions.

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